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Udo Hetzel,[a]* Tarja Sironen,[b] Pasi Laurinmäki,[c] Lassi Liljeroos,[c] Aino Patjas,[b] Heikki Henttonen,[d] Antti Vaheri,[b] Annette Artelt,[e] Anja Kipar,[a,f]* Sarah J. Butcher,[c] Olli Vapalahti,[a,b,g] Jussi Hepojoki[b]

Veterinary Pathology, Department of Veterinary Basic Sciences, Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland[a]; Department of Virology, Haartman Institute, University of Helsinki, Helsinki, Finland[b]; Department of Biosciences and Institute of Biotechnology, University of Helsinki, Helsinki, Finland[c]; Finnish Forest Research Institute, Vantaa, Finland[d]; Institute of Veterinary Pathology, Justus-Liebig-University, Giessen, Germany[e]; Veterinary Pathology, School of Veterinary Science and Department of Infection Biology, Institute of Global Health, University of Liverpool, Liverpool, United Kingdom[f]; Department of Virology and Immunology, HUSLAB, Hospital District of Helsinki and Uusimaa, Helsinki, Finland[g]

The comment letter by Dr. Bodewes and colleagues provides an update on the phylogeny of arenaviruses (Avs) that we have termed boid inclusion body disease-associated (BIBD) Avs in our recent publication (1). It shows interesting relatedness between the University of Helsinki virus (UHV), an arenavirus that we isolated in cell culture from a boa with BIBD and characterized in detail, and boa Av NL B3, a virus that their group detected by sequencing (2). When we submitted our manuscript early in 2013, we were not aware of the forthcoming publication by the Dutch group, and since the virus sequences were not available in GenBank at that stage (the sequences were released on 22 May 2013), we were unfortunately not able to undertake a phylogenetic comparison. Fortunately, this is now possible. Interestingly, the two viruses show a 38% divergence in GPC at the amino acid level, which is considerable for the surface proteins that are responsible for neutralization. In contrast to that, as Bodewes and colleagues point out in their comment, higher homologies are seen in the other genes, alongside a discordant phylogenetic relatedness within the UHV/NL B3/Golden Gate virus (GGV) group. This suggests intracistronic/segment recombination (and/or reassortment).

Bodewes et al. also express the opinion that the UHV and NL B3 viruses share a "number of the characteristics used by the International Committee on Taxonomy of Viruses (ICTV) to include different isolates of arenaviruses to the same species." While the emergence of new snake arenaviruses may require that the ICTV criteria are refined in the future, Bodewes et al.'s interpretation of the current ICTV criteria may be arguable. The first ICTV criterion is "an association with a specific host species or group of species" (3) and, even though BIBD Avs have been isolated mainly from boa constrictors (the California Academy of Sciences virus was from *Corallus annulatus* [4]), this species has so far not been established as the reservoir host of these viruses. The second ICTV classification criterion is "presence in a defined geographical area" (3), and although the UHV isolate and the boa Av NL B3 sequence originate from boa constrictors that live in neighboring countries (Germany and the Netherlands), the origins of the viruses remain unknown and may even be on another continent. The third demarcation criterion, "etiological agent (or not) of disease in humans" (3), remains to be studied and may, indeed, be irrelevant in this context. In order for the fourth criterion of the ICTV, "significant differences in antigenic cross-reactivity, including lack of cross-neutralization activity where applicable" (3), to be fulfilled, isolation and antibody production against the other BIBD Av needs to be performed. This is technically possible with the methods and tools that we have described (1). The final ICTV criterion, "significant as sequence difference from other species in the genus" (3), is not a very exact expression. Our interpretation is that the observed divergences in the GPC (38%) amino acid sequences of UHV and Boa Av NL B3 are significant enough to consider these viruses two different species.

It appears also possible that both the viruses discussed in this letter would be considered variants of GGV, described by Stenglein et al. (4), since both of these viruses are approximately equally distant from GGV. In this scenario, however, similar challenges regarding the assessment of the demarcation criteria would arise. Since several novel BIBD Avs were isolated or detected within the last year, the phylogenetic tree of the family *Arenaviridae* is likely to be altered dramatically in the near future. Thus, in our opinion, any decision on the taxonomic nomenclature of these novel viruses at the species level appears premature at this stage.

REFERENCES

1. **Hetzel U, Sironen T, Laurinmaki P, Liljeroos L, Patjas A, Henttonen H, Vaheri A, Artelt A, Kipar A, Butcher SJ, Vapalahti O, Hepojoki J.** 2013. Isolation, identification, and characterization of novel arenaviruses, the etiological agents of boid inclusion body disease. J. Virol. **87:**10918–10935. http://dx.doi.org/10.1128/JVI.01123-13.
2. **Bodewes R, Kik MJ, Raj VS, Schapendonk CM, Haagmans BL, Smits SL, Osterhaus AD.** 2013. Detection of novel divergent arenaviruses in boid snakes with inclusion body disease in The Netherlands. J. Gen. Virol. **94:** 1206–1210. http://dx.doi.org/10.1099/vir.0.051995-0.
3. **King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ (ed).** 2012. Virus taxonomy: classification and nomenclature of viruses. Ninth report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, San Diego, CA.
4. **Stenglein MD, Sanders C, Kistler AL, Ruby JG, Franco JY, Reavill DR, Dunker F, Derisi JL.** 2012. Identification, characterization, and in vitro culture of highly divergent arenaviruses from boa constrictors and annulated tree boas: candidate etiological agents for snake inclusion body disease. mBio 3(4):e00180–12. http://dx.doi.org/10.1128/mBio.00180-12.

Address correspondence to Jussi Hepojoki, jussi.hepojoki@helsinki.fi.

* Present address: Udo Hetzel and Anja Kipar, Vetsuisse Faculty, University of Zürich, Zürich, Switzerland.

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